

pH measurement in Escherichia coli fermentation with EasyFerm Food

Uninhibited cell growth due to new electrolyte Foodlyte in the pH sensor EasyFerm Food

Industry: Biotechnology

Application: Fermentation / Protein Synthesis

Hamilton product: EasyFerm Food VP, EasyFerm Plus VP

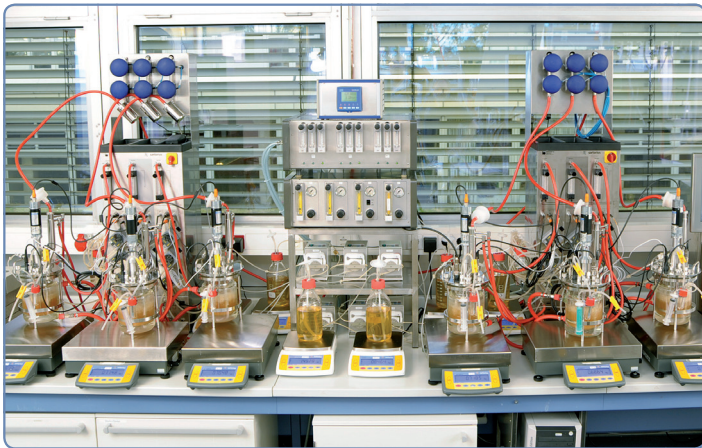


Figure 1: Multifermeter BIOSTAT® Qplus 6 with periphery from the company Sartorius Stedim Biotech

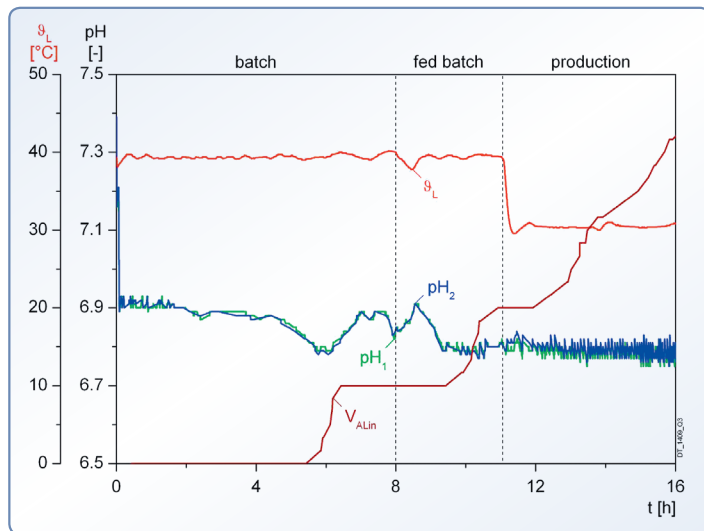


Figure 2: Comparison of pH1 (EasyFerm Food 160 VP, S/N: 1003) with pH2 = EasyFerm Plus 160 K8.

θ_L : Temperature of the liquid phase

V_{ALin} : Added volume of ammonia for base titration

In fermentations, it is very important that the cells have optimal conditions for growth and protein synthesis. To ensure this, none of the components should pose a threat to the micro organisms used in fermentation. Electrolyte outflow from certain pH electrodes could possibly affect microorganisms used in fermentation in an undesired way.

One important design goal of the new Hamilton pH sensor EasyFerm Food is to avoid any effect on micro organisms in fermentation. This is achieved by the new electrolyte Foodlyte, which consists only of components compatible with food.

The EasyFerm Food electrode with the new electrolyte Foodlyte was tested by MDT* and passed the cytotoxicity test in reactor volumes down to 500 ml (worst case scenario). Therefore, this electrode is suitable for applications such as fermentation, where the risk of cell growth reduction or cell death must be excluded.

This application note is based on experiments that Dipl. Ing. Frithjof Tatge performed at the Hamburg University of Applied Sciences.

Measurement Equipment

The practical performance of the EasyFerm Food VP was tested in a Biostat® Q PLUS 6 Multifermeter with periphery from Sartorius Stedim Biotech. The system consists of 6 glass reactors (1l) which are equipped with DO-, temperature and pH-sensors (one EasyFerm Plus K8 and one EasyFerm Food VP each). A gas mixing station ensures the required ratio of oxygen to nitrogen and a pump station allows Fed Batch operation. The measurement of oxygen and CO₂ is accomplished with sensors from BlueSens. The two supply units were connected to gas and cold water supplies for the reactors, stirrers, and sensors

for DO, pH (EasyFerm Plus K8 or EasyFerm Food VP), and temperature.

Measuring with EasyFerm Food/Plus

Control of the exact pH-values is essential for the cultivation of Escherichia coli bacteria and production of proteins. Throughout the course of the procedure a constant pH value of the media at around 6.8 was obtained by addition of ammonia or phosphoric acid. The pH adjustment was based on values from the EasyFerm Plus electrode in each reactor. EasyFerm Food electrodes were run in parallel to test the suitability of the new electrolyte Foodlyte which consists only of components compatible to fermentation processes.

In a first step the pH sensors were calibrated in Hamilton buffers pH 4.00 and pH 7.01. The sensors were then mounted into the medium filled reactor and autoclaved at 121 °C for 15 minutes. After sterilization, the reactor content was cooled to 37 °C and pH sensors were recalibrated.

In the “batch phase” of the reaction a constant oxygen flow (1.5 l/h) was led into the medium until the substrate (glucose) was consumed. In the following “fed batch phase” glucose was added and an oxygen air stream was fed into the reactor. Once the cell density of the E. coli bacteria reached 15 g/l, IPTG (Isopropyl-β-D-thiogalactopyranoside) was added to the mixture. This induced the “production phase” and the temperature decreased to between 31 and 25 °C, while the stirring speed was increased up to 1000 rpm. In this phase protein was produced and harvested for up to 20 h. At the end of the reaction the whole medium was autoclaved for a second time.

Result

EasyFerm Food delivered pH values of high accuracy and reproducibility that were comparable to those of the established and proven EasyFerm Plus. All calibrations were in the accepted range.

Both EasyFerm Plus and EasyFerm Food have successfully shown their ability to be used in fermentation processes. The fact that EasyFerm Food was designed and certified for excellent biocompatibility makes it a first choice in biotechnological, pharmaceutical and food applications.

Acknowledgement

We would like to thank Prof. Dr. Dipl. Ing. Luttmann** for his collaboration and advice on this project. Furthermore we like to thank Dipl. Ing. Frithjof Tatge for work done on this project as a part of his diploma thesis and Dip. Ing. Scheffler** for his technical support on the setup of the multifermenter BIOSTAT® QPLUS 6.

* MDT = Medical Device Testing GmbH Ochsenhausen, Germany
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** Hamburg University of Applied Sciences

User benefits of EasyFerm Food

- ▶ No inhibited cell growth due to the food safe Foodlyte gel reference electrolyte
- ▶ Cleanability tested and certified according to EHEDG criteria
- ▶ Biocompatibility tested and certified by MDT
- ▶ Suitable for small reactor volumes down to 500 ml
- ▶ Measurement with high accuracy and reproducibility

Technical data EasyFerm Food VP

Range of measurement	pH 0 to 14
Zero point	0 ± 20 mV
Sensitivity	57 to 59 mV / pH at 25 °C
Operation temperature	0 to 135 °C
Pressure range	0 to 6 bar
Temperature sensor	Pt100
Measurement principle	Potential measured against reference
Shaft material	Glass
Membrane glass	Hamilton type “HB” glass
O-ring material	EPDM (FDA approved)
Electrolyte	Pressurized Foodlyte
Diaphragm material	HP Coatramic
Autoclavable	Yes
CIP	Yes
Steam Sterilizable	Yes

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